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Oral Iron Replacement Normalizes Fibroblast Growth Factor 23 in Iron Deficient Patients with Autosomal Dominant Hypophosphatemic Rickets

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Abstract

Autosomal dominant hypophosphatemic rickets (ADHR) is caused by mutations impairing cleavage of fibroblast growth factor 23 (FGF23). *FGF23* gene expression increases during iron deficiency. In humans and mice with the ADHR mutation, iron deficiency results in increased intact FGF23 concentrations and hypophosphatemia.

We conducted a prospective open label pilot clinical trial of oral iron replacement over 12 months in ADHR patients to test the hypothesis that oral iron administration would normalize FGF23 concentrations. Eligibility criteria included: *FGF23* mutation; and either serum iron <50 mcg/dl; *or* serum iron 50-100 mcg/dl combined with hypophosphatemia and intact FGF23 >30 pg/ml at screening. Key exclusion criteria were kidney disease and pregnancy. Oral iron supplementation started at 65 mg daily and was titrated based on fasting serum iron concentration. The primary outcome was decrease in fasting intact FGF23 by ≥20% from baseline.

Six adults (3 male, 3 female) having the *FGF23-R176Q* mutation were enrolled; 5 completed the 12-month protocol. At baseline 3/5 subjects had severely symptomatic hypophosphatemia (phosphorus <2.5 mg/dl) and received calcitriol with or without phosphate concurrent with oral iron during the trial.

The primary outcome was met by 4/5 (80%) subjects all by Month 4, and 5/5 had normal intact FGF23 at month 12. Median (minimum, maximum) intact FGF23 concentration decreased from 172 (20, 192) pg/ml at baseline to 47 (17, 78) pg/ml at Month 4 and 42 (19, 63) pg/ml at Month 12. Median ferritin increased from 18.6 (7.7, 82.5) ng/ml at baseline to 78.0 (49.6, 261.0) ng/ml at Month 12. During iron treatment, all 3 subjects with baseline hypophosphatemia normalized

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serum phosphorus, had markedly improved symptoms, and were able to discontinue calcitriol and phosphate.

Oral iron repletion normalized FGF23 and phosphorus in symptomatic, iron-deficient ADHR subjects. Thus, the standard approach to ADHR should include recognition, treatment, and prevention of iron deficiency.

Introduction

Autosomal dominant hypophosphatemic rickets (ADHR) is a rare bone disease caused by mutations in a cleavage recognition motif, RXXR, that impair cleavage of fibroblast growth factor 23 (FGF23) [1,2]. Individuals with FGF23 mutations causing ADHR have a variable clinical presentation, with delayed penetrance and incomplete penetrance of the phenotype [3]. Children with ADHR mutations can be either normophosphatemic without evidence of bone disease, or may present with hypophosphatemic rickets, clinically appearing similar to the more common X-linked hypophosphatemia. Previously normophosphatemic children harboring ADHR mutations may then develop late onset hypophosphatemic osteomalacia as adolescents or adults [3–5] often with severe symptoms that mimic the presentation of tumor induced osteomalacia. This late presentation accounts for about half of the symptomatic patients in the kindreds we have studied. Both children and adults with ADHR also spontaneously normalize serum FGF23 and serum phosphorus, or go through cycles of alternating hypophosphatemia and normophosphatemia [4,5].

We previously demonstrated that the clinical phenotype of ADHR is temporally related to the presence of iron deficiency [5]. ADHR mutant mice fed a standard diet, do not become iron deficient and do not manifest elevations of plasma intact FGF23 or hypophosphatemia [6]. When iron depleted, the *FGF23* mRNA expression of both ADHR mutant mice and of wild-type mice increases, resulting in increased plasma C-terminal FGF23 concentrations [6]. However, the wild type mice maintain normal intact FGF23 through cleavage, while the ADHR mutant mice develop elevated intact FGF23 due to resistance of the protein to cleavage. Thus the wild type mice remain normophosphatemic, while the ADHR mutant mouse develops hypophosphatemia secondary to elevated intact FGF23 [6]. Similarly, in cross-sectional analysis there was an inverse relationship between serum iron and C-terminal FGF23 concentrations in both healthy control subjects [5,7] and subjects with ADHR mutations [5], but between serum iron and intact FGF23 concentrations only in those with ADHR mutations. Thus, FGF23-induced hypophosphatemia corresponded to times of low serum iron concentrations in ADHR subjects [5], providing an explanation for the observed waxing and waning phenotype.

Hypophosphatemic osteomalacia in ADHR is conventionally managed similarly to that in X-linked hypophosphatemia (XLH), using oral phosphate salts and activated forms of vitamin D, such as calcitriol [3,8]. While this strategy results in symptomatic improvement, limitations include gastrointestinal side effects, and risks of hyperparathyroidism and nephrocalcinosis. In addition, treatment with calcitriol and phosphate increase FGF23 concentrations[9–11], even in ADHR subjects[4]. However, if iron deficiency is driving FGF23 elevations in ADHR, iron supplementation would be a mechanistically more

appropriate treatment. In a retrospective case report, Kapelari et al described a child with ADHR that was able to withdraw from treatment with calcitriol and phosphate having normal serum phosphorus after achieving iron sufficiency[12]. We conducted a prospective open label pilot clinical trial to test the hypothesis that oral iron administration would normalize FGF23 concentrations in ADHR subjects.

Methods

Study design:

We conducted a prospective open label single center pilot clinical trial of oral iron administration in subjects with ADHR (ClinicalTrials.gov NCT02233322). The primary outcome was a decrease in intact FGF23 concentrations ≥20% from baseline. This study was approved by the Indiana University Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All subjects signed informed consent before participation.

Study subjects:

Potential subjects from families with ADHR were screened for *FGF23* mutations. Eligible subjects included individuals age 2 through adulthood, with a missense mutation in the *FGF23* gene that leads to an amino acid change in either arginine 176 or 179, consistent with ADHR. Subjects were also required to have at the screening visit either: serum iron concentrations < 50 mcg/dl (with or without hypophosphatemia); *or* if iron was between 50 and 100 mcg/dl, also be hypophosphatemic for age and have intact FGF23 >30 pg/ml. Intact FGF23 concentrations using the Kainos assay of >30 pg/ml (being above the normal mean) during hypophosphatemia have been proposed as consistent with an FGF23-mediated hypophosphatemia [13]. Subjects were allowed to be receiving calcitriol and/or phosphate as therapy for ADHR concurrently, if they were willing to let the investigators decrease or stop the dosing based on laboratory values if the serum phosphorus was improving. Exclusion criteria were terminal illness, severe end organ disease, malignancy, pregnancy or planned pregnancy. Chronic kidney disease with estimated GFR <45 ml/min/1.73m^2 (calculated using the MDRD formula for adults) was also excluded because GFR in this range are associated independently with elevated FGF23 concentrations which could obscure the results [14,15].

Intervention:

Subjects were treated with oral iron supplements in a standardized approach using ferrous sulfate 325 mg tablets (containing 65 mg elemental iron). Subjects were instructed not to take the iron at the same time as milk, caffeine, antacids, calcium supplements, calcitriol, or phosphate, to avoid confounding by impaired absorption. Subjects were allowed to take the iron with food to decrease gastrointestinal side effects and to improve compliance. When subjects required more than one pill, the dose was split into 2 or 3 doses in a day. On days when fasting samples were obtained, subjects were instructed to not take iron until after phlebotomy was completed. Stool softeners (e.g. docusate sodium) were allowed in case of constipation.

Iron doses were titrated upward according to tolerance (such as to gastrointestinal symptoms) and the following algorithm. If the initial serum iron concentration was between 50 and 100 mcg/dl, the dose started at 65 mg elemental iron daily for the first month. If the starting iron concentration was <50 mcg/dl, dosing also started at 65 mg elemental iron daily for one week and then increased to 65 mg elemental iron twice a day. At subsequent visits the following titration occurred. If the serum iron remained <50 mcg/dl, the elemental iron dose was increased by 65 mg daily and levels were rechecked in one month. If iron was between 50 to <100 mcg/dl, the iron dose was increased by 65 mg daily, unless the iron level had increased by more than 50 mcg/dl from the previous visit. If the iron level was 100 to <150 mcg/dl, the dose remained the same. If the iron level was 150 to <170 mcg/dl, the dose was decreased by 65 mg daily. If the iron level was >170 mcg/dl (>80% of the upper normal limit), iron dosing would be stopped until the next scheduled measurement. Prior to any dose increases, compliance with the regimen was assessed by discussion with the subject.

Subjects receiving phosphate and calcitriol at enrollment were allowed to continue. However those not already receiving phosphate or calcitriol were not initiated on these agents unless there was evidence of hypophosphatemia with symptomatic osteomalacia (e.g. bone pain, pseudofractures, etc.). Calcitriol and phosphate doses were tapered or stopped if serum phosphorus was in the normal range.

Measurements:

All laboratory measurements were made using fasting samples. The following measurements were conducted in the Indiana University Health Pathology Laboratory: hemoglobin and serum iron, total iron binding capacity (TIBC), ferritin, calcium, creatinine, phosphorus, and alkaline phosphatase. Percent iron saturation was assessed as the serum iron ÷ TIBC. Subjects were classified as iron deficient based on ferritin < 30 ng/ml or iron saturation < 20%[16,17]. FGF23 was measured in EDTA-plasma, to ensure no assay interference by iron levels (due to binding of divalent cations by EDTA. Intact FGF23 concentrations was measured using an ELISA, which detects only intact FGF23 (Kainos Laboratories, Inc, Tokyo, Japan), and C-terminal FGF23 (detecting the combination of intact FGF23 and C-terminal fragments) was measured using the Immutopics C-terminal FGF23 ELISA (Immutopics International, San Clemente, CA). Coefficient of variation (CV) for the intact FGF23 ELISA is 4.4% with a lower limit of detection of 3 pg/ml. CV of the C-terminal FGF23 ELISA is 4% and the lower limit of detection is 1.5 RU/ml [5].

Statistical Analysis.

This was a pilot single-arm clinical trial of the rare disease ADHR. For the primary outcome, the percent change of intact FGF23 at each time point was assessed compared to the average of the screening and baseline values. The primary endpoint was evaluated beginning at 3-months after starting iron supplementation. A subject was considered as having responded if intact FGF23 decreased by 20% or more from the baseline level. The original planned sample size N=8 was chosen based on feasibility considerations due to the rarity of ADHR. Based on clinical considerations from ADHR experts, the treatment would be considered as successful if 4 or more (4+) patients respond out of N=8. With N=8, if the true response rate is 60%, the probability of declaring a success was 0.83. On the other hand,

if the true response rate is no more than 20%, the probability of declaring a success was 0.0563. For analyses of other outcomes, summary statistics such as median (minimum, maximum) for continuous variables and count (percentage) for categorical variables were generated.

Results

Fifty-two patients from kindreds with ADHR were screened in 2016 and 2017. Of these, 29 were negative for mutations and 23 had FGF23 mutations. Of those with mutations, 15 subjects were excluded based on biochemical criteria at screening (all 15 had normal serum iron: 7 having serum iron > 100 mcg/dl and 8 having serum iron between 50 and 100 mcg/dl with normal serum phosphorus). Eight subjects met laboratory inclusion criteria; and two declined to participate in the study protocol. Thus, six adults (3 male, 3 premenopausal female) having the FGF23-R176Q mutation were enrolled with a median age of 44 years (ranging from 25 to 65 years).

Consistent with the eligibility criteria, differences in screening biochemistries were observed between those with mutations who failed screening and those that were enrolled. Mean (± SD) intact FGF23 in those with mutations that screen failed was 46 ± 18 pg/ml (median 49) versus 115 ±70 in enrolled subjects (p=0.08). Serum phosphorus was 3.2 ± 0.6 mg/dl (median 3.2) vs 2.1 ± 0.4 (median 2.2) for screen failed versus enrolled subjects, respectively (p<0.01). Two screen failed subjects were mildly hypophosphatemic (2.2 and 2.4 mg/dl). Serum iron was 102.7 ± 29.9 mcg/dl (median 112) versus 43.6 ± 21 mcg/dl (median 48) for screen failed versus enrolled subjects, respectively (p<0.01). C-terminal FGF23, ferritin and hemoglobin were not measured at screening.

Five subjects completed the 12-month protocol, while one subject that was normophosphatemic at baseline was lost to follow-up after month 3. Of the 5 completing subjects, baseline symptoms included bone pain (4/5), back pain (4/5), weakness (3/5), joint pain (4/5), joint stiffness (4/5), leg bowing (1/5) and tooth abscesses (1/5). Laboratory values at baseline and throughout the trial are summarized in Table 1.

At screening 4/5 subjects were hypophosphatemic (phosphorus <2.5 mg/dl). Severe symptoms of hypophosphatemia were present in 3/5 subjects, requiring treatment with calcitriol, with or without oral phosphate (1 beginning 3 years prior to enrollment, 1 beginning at the baseline visit, 1 beginning at month 2). These three subjects also had low ferritin (<30 ng/ml), low iron saturation (<20%) along with low hemoglobin (<13.4 g/dl) consistent with iron deficiency and anemia at baseline, and two of which also had low mean cell hemoglobin (MCH) and mean cell volume (MCV) (Table 1). Specific etiologies for iron deficiency were not available. No subjects were receiving iron supplementation prior to enrollment.

By the end of the trial, daily elemental iron doses were titrated to 130 mg (1/5), 195 mg (1/5) and 260 mg (3/5). Median (minimum, maximum) serum iron concentrations had increased from 69 (22, 83) ug/dl at baseline to 82 (43, 117) ug/dl at Month 12. Median serum ferritin had increased from 18.6 (7.7, 82.5) ng/ml at baseline to 78.0 (49.6, 261.0)

ng/ml at Month 12. No subjects met the protocol's criteria for iron dose decrease (serum iron levels >150 mcg/dl).

The primary outcome of decreasing plasma intact FGF23 ≥20% from baseline was attained in 4/5 subjects, but all subjects had intact FGF23 in the normal range (<70 pg/ml) [18] at Month 12 (Figure 1). Similarly, 4/5 subjects decreased C-terminal FGF23 concentrations at any point during the study, and all subjects had C-terminal FGF23 concentrations within the normal range (<180 RU/ml) [5,19] at Month 12 (Figure 1). Subject 4 (labeled in green in the figures) increased intact FGF23 during the first 2 months. This subject was also receiving both calcitriol and phosphate which we have previously demonstrated to increase plasma FGF23 even in ADHR [5]. However, this subject also subsequently decreased intact FGF23 into the normal range after iron therapy and stopped calcitriol and phosphate.

Serum phosphorus increased from 2.1 (<1.0, 2.7) mg/dl at baseline to 2.8 (2.5, 4.0) mg/dl at Month 12 (Figure 2). One subject with severely symptomatic hypophosphatemia had been treated with calcitriol and phosphate for 3 years before enrolling in the trial and had normal alkaline phosphatase at the time of screening. Only two subjects had elevated serum alkaline phosphatase at baseline which normalized in both during the trial (Figure 2). Serum calcium remained normal throughout the trial. Of the three subjects that were taking calcitriol/phosphate, all normalized serum phosphorus and were able to discontinue calcitriol and phosphate (1 at Month 3, 1 at Month 4, and 1 at Month 9) and all remained normophosphatemic through Month12, with clinical improvement in symptoms.

Adverse events were limited. Adverse events rated as mild included one subject with constipation and one with influenza. One patient had increased bone pain in the hip rated as moderate, and mild lateral epicondylitis bilaterally. One patient had an episode of diverticulitis rated as moderate in severity. Only the constipation was considered related to iron supplementation. There were no serious adverse events.

Discussion

This trial confirms the clinical importance of iron to the mechanism of ADHR. We previously demonstrated that the clinical phenotype of ADHR waxed, waned, and in some cases resolved and recurred [3]. We subsequently found in observational studies that this clinical change was related to changes in plasma intact FGF23 and serum phosphorus[4], which corresponded to whether individuals with ADHR were iron deficient versus iron sufficient[5]. In this prospective pilot clinical trial we demonstrate clearly that intervention with oral iron supplementation among iron deficient subjects with ADHR normalized plasma intact FGF23, C-terminal FGF23, and serum phosphorus.

By the end of the trial subjects had improvements in iron status which was most readily demonstrated by changes in serum ferritin, and all subjects had normal FGF23 concentrations and serum phosphorus. Although 4/5 subjects decreased intact FGF23 by ≥20% from baseline, the most obvious biochemical responses occurred in the three subjects with elevated FGF23, frank hypophosphatemia, and iron deficiency at baseline (as indicated by low ferritin, iron saturation and hemoglobin) [16], who normalized FGF23, phosphorus

and ferritin during the study. These subjects were also able to discontinue calcitriol and phosphate and maintain normophosphatemia after iron repletion. This is consistent with data from our original observational study describing the relationship between iron and FGF23 in ADHR[5]. In that study a subgroup of 15 ADHR subjects had longitudinal samples showing an inverse relationship between serum iron and C-terminal FGF23 over time, though there was no available information regarding iron supplementation[5]. Similar to patients in our prospective study, in a retrospective case report, a child with ADHR who was supplemented with iron was able to withdraw from treatment with calcitriol and phosphate after achieving iron sufficiency during oral iron treatment[12].

This study has clinically important consequences for the management of patients with ADHR. Management of FGF23-mediated hypophosphatemia with calcitriol and phosphate has risks of clinically important adverse events including nephrocalcinosis and hypercalcemic hyperparathyroidism. Management of iron deficiency with iron supplementation would be clinically indicated even in the absence of any effect on FGF23. We demonstrate that oral iron administration addressed both issues, although symptomatic patients may benefit from treatment with calcitriol and phosphate until iron stores are replete. It should be noted that not all of the subjects in this study met strict criteria for iron deficiency. This is in accord with our prior study where some subjects had low normal iron studies, but still had hypophosphatemia [5].

However our prior ADHR studies did not include ferritin, which may be a better marker of iron deficiency than serum iron[16,17]. While not all of the responders had low serum iron at baseline, all three had low ferritin. In contrast, the two subjects with normal ferritin at baseline consistent with more normal iron stores, both increased ferritin above 200 ng/ml at the end of the study and had transient iron saturation >45%, with less impact on FGF23 and phosphorus (though both had normal FGF23). This supports our hypothesis that it is iron stores rather than serum iron itself that is responsible for the relationship with FGF23. Thus, FGF23 and phosphate normalized in patients with iron deficiency at baseline, but it did not change in those with iron sufficiency at baseline.

While our titration protocol was based on serum iron, it may be more appropriate to titrate iron based on serum ferritin concentrations. Based on the results of this study, we would recommend that an important goal of treatment with iron, even in ADHR, is treatment of the iron deficiency, with the ancillary benefit of improving FGF23 and phosphate. There is a risk for iron overload, especially if treating iron sufficient patients. Thus, it is important to monitor iron stores during repletion with iron to avoid iron excess and its associated complications. After initial repletion, some subjects may still require smaller supplementation to ensure appropriate intake of iron to maintain normal stores, which should be individualized to meet needs according to sex, age and whether there are ongoing iron losses. We hypothesize that maintaining mid normal iron stores might resolve or completely prevent hypophosphatemia in patients with ADHR, without the risks of calcitriol or phosphate therapy. The long-term efficacy and safety of this approach require further study.

We previously published a cross sectional analysis of serum iron in patients with X-linked hypophosphatemia (XLH) using samples obtained prior to starting treatment with calcitriol or phosphate[19]. As opposed to the relationship seen in ADHR, the relationship of serum iron to FGF23 in XLH was more similar to that of healthy controls. In this regard there was an inverse relationship between C-terminal FGF23 and iron in patients with ADHR, those with XLH, and in healthy controls, but an inverse relationship between intact FGF23 and iron was only present in ADHR [19]. There was no relationship between intact FGF23 and serum iron in XLH. Thus, we would expect that iron supplementation to optimize iron stores would not be useful in managing serum phosphorus in XLH, while our data suggests that it could be curative in ADHR, at least for some patients.

Limitations of our study include the overall small number of subjects, and the lack of bone biopsy data to confirm effects on osteomalacia. Due to the waxing and waning nature of the clinical phenotype in ADHR, most subjects with mutations did not meet inclusion criteria resulting in a small study population. However, our results support our hypothesis that the clinical phenotype in ADHR results from the consequences of iron deficiency in the setting of FGF23 mutations. The strengths of our study include the prospective nature and the robust effects, especially among those subjects with low hemoglobin, iron deficiency, and hypophosphatemia at baseline.

However to complicate the relationship of iron and FGF23, some types of iron infusions are known to precipitate acute elevations of intact FGF23 with resulting severe hypophosphatemia when administered to iron deficient patients without ADHR[20–23]. The mechanism for this effect is unclear, but as the frequency appears to vary with different types of iron infusions, the prevailing hypothesis is that the carbohydrate moiety of the iron preparation may alter FGF23 cleavage on the background of increased gene expression in the setting of iron deficiency. When patients persist in iron deficiency, FGF23 gene expression would remain elevated and subsequent iron infusions could still induce additional spikes in circulating intact FGF23 and resulting hypophosphatemia. There are several reports describing such resulting hypophosphatemic osteomalacia after iron infusion, particularly with ferric polymaltose and ferric carboxymaltose [24–27].

Thus, for safety reasons, we chose to focus on oral iron supplementation to avoid the risk of acutely worsening intact FGF23 and hypophosphatemia. Such an effect might be worse in a patient with ADHR, having a mutation that already impairs cleavage of the FGF23 produced from one allele, if iron infusions were to also impair the cleavage of FGF23 produced from the normal allele. Indeed, we would recommend avoiding intravenous iron in ADHR patients. During our study one subject had a temporary large increase in intact FGF23 one month after starting iron which had normalized by 2 months of iron supplementation. This subject was also taking calcitriol and phosphate prior to the trial, which also increase FGF23 [9–11]. We only used oral ferrous sulfate in our trial. Thus, we cannot comment on comparative effectiveness of oral ferric compounds.

Recently, an anti-FGF23 antibody, burosumab, was FDA approved for treatment of in XLH[28,29]. Burosumab use in ADHR has not been studied, is not FDA approved, and is outside the scope of our study. Given the significant expense of burosumab we would not

recommend its off-label use. Even if a patient with ADHR responded to burosumab, they would still clinically need to have the underlying iron deficiency addressed, while conversely treating a patient's iron deficiency may simultaneously resolve the hypophosphatemia much less expensively. A further concern is that an ADHR patient might be at higher risk for hyperphosphatemia from burosumab if they spontaneously normalized their FGF23 production due to restoration of normal iron stores.

Conclusion

This prospective trial confirmed our previous observational study findings. The clinical phenotype of ADHR is directly related to iron status. Treatment with oral iron to normalize iron stores in iron deficient patients with ADHR is able to normalize plasma intact FGF23 and serum phosphorus, allowing patients to stop or even avoid treatments with calcitriol or phosphate. Oral iron supplementation to maintain sufficient (but not excessive) iron stores may be able to maintain normophosphatemia. The standard approach to ADHR should include recognition, treatment, and prevention of iron deficiency.

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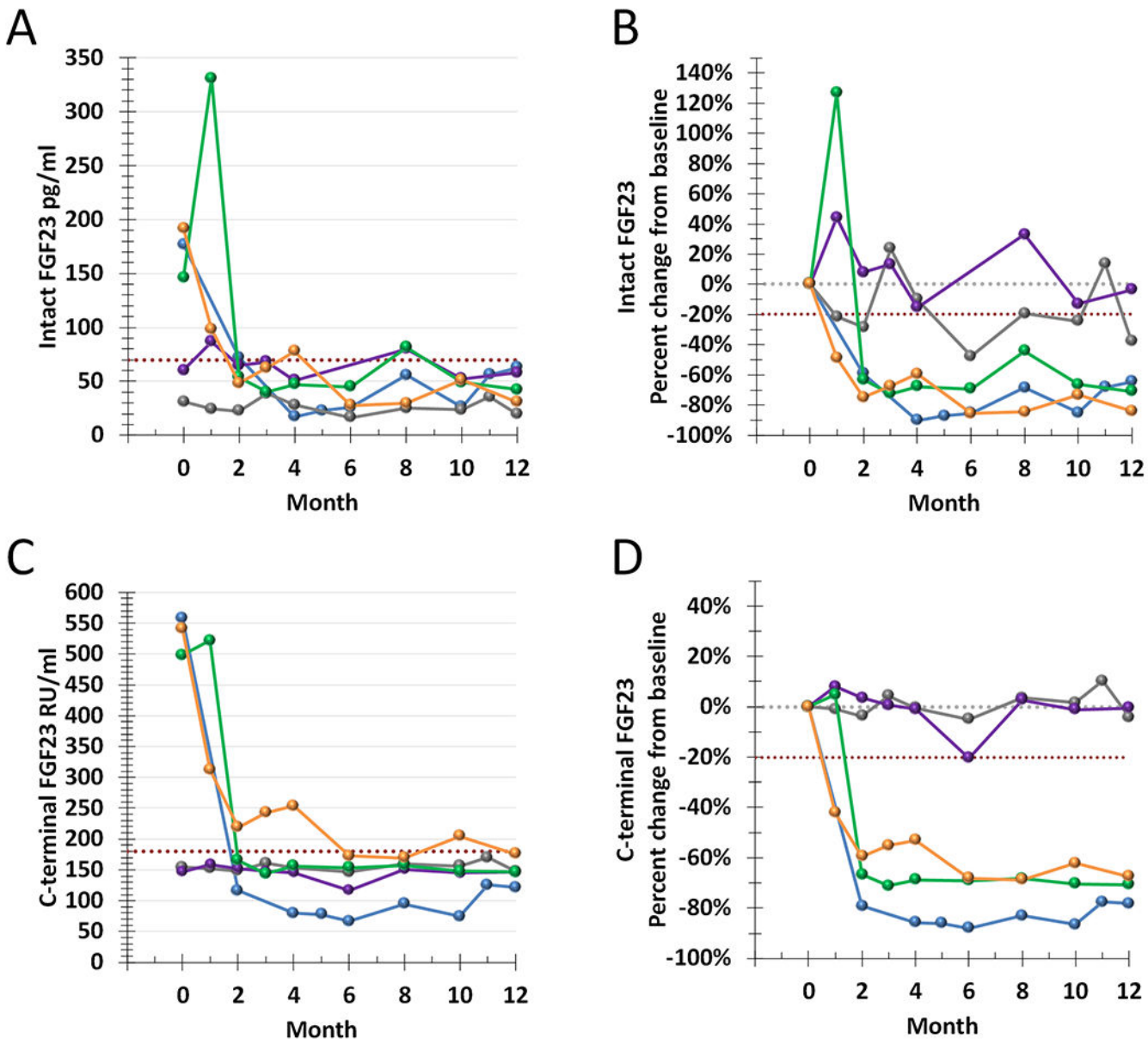

Figure 1.

FGF23 during oral iron supplementation. Intact FGF23 (A) and percent change from baseline in intact FGF23 (B), along with C-terminal FGF23 (C) and percent change from baseline in C-terminal FGF23 concentrations (D). Each line represents an individual subject. Zero represents the baseline visit. The red dotted line represents the upper limit of normal for intact FGF23 (A) and C-terminal FGF23 (C), or a 20% decrease from baseline (B and D). The subject in green was on treatment with calcitriol and phosphate for 3 years prior to enrollment and stopped at Month 3. The subject in yellow started calcitriol at the baseline visit and stopped at Month 4. The subject in blue started calcitriol at Month 2 and stopped Month 9.

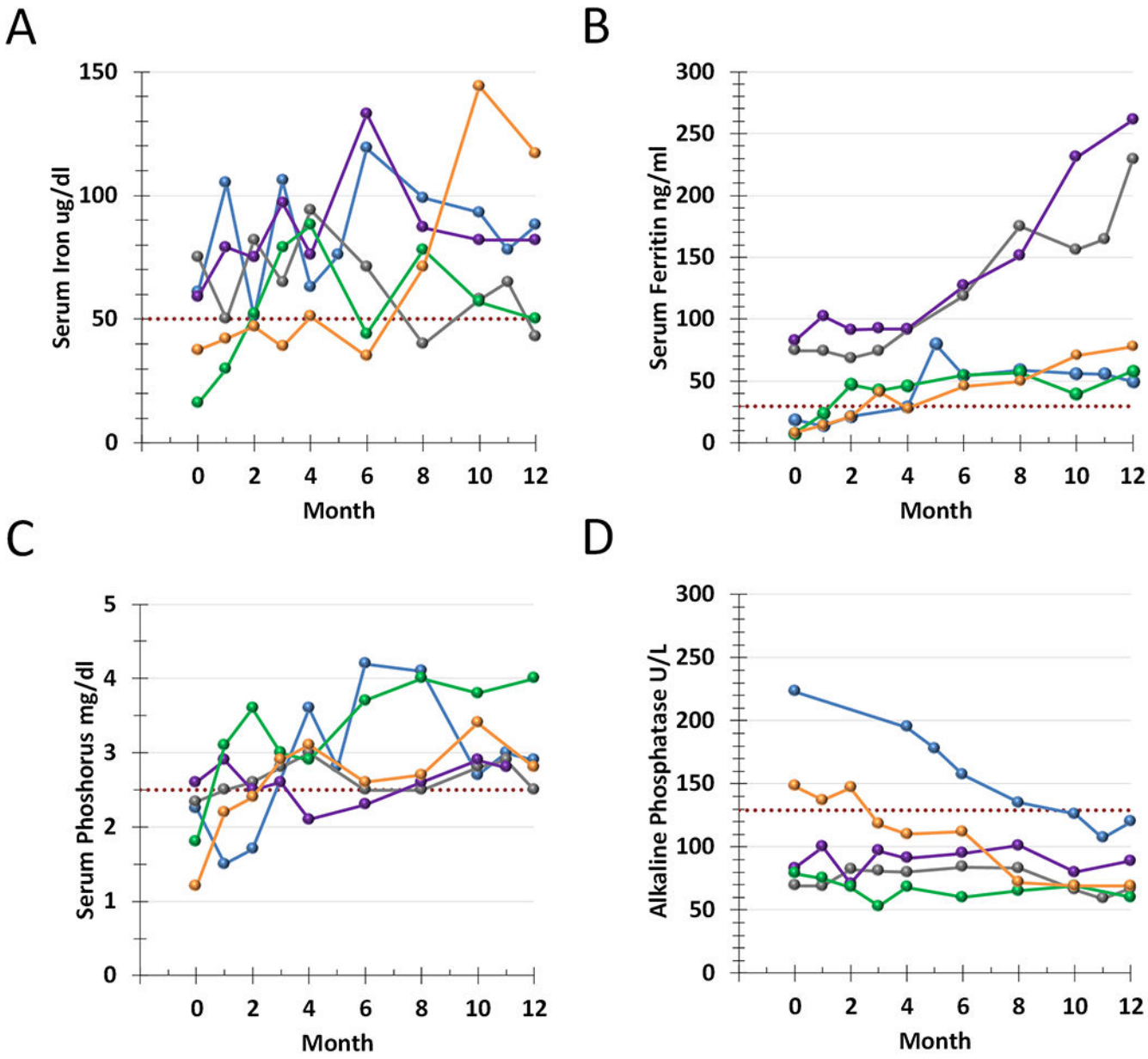

Figure 2.

Serum iron (A), ferritin (B), phosphorus (C) and alkaline phosphatase (D) during oral iron supplementation. Each line represents an individual subject. Zero represents the baseline visit. The red dotted line represents the lower limit of normal for serum iron (A), ferritin (B), and phosphorus (C), and the upper limit of normal for alkaline phosphatase (D). The subject in green was on treatment with calcitriol and phosphate for 3 years prior to enrollment and stopped at Month 3. The subject in yellow started calcitriol at the baseline visit and stopped at Month 4. The subject in blue started calcitriol at Month 2 and stopped Month 9.

Table 1.

Baseline biochemistries for individual subjects

Subject ID	Sex	Age yrs	Graph color	Phosphorus	Calcium	Creatinine	Alkaline phosphatase	Iron	Iron saturation	Ferritin	Hemoglobin	Mean cell hemoglobin (MCH)	Mean cell volume (MCV)	Intact Fgf23	C-terminal FGF23
				2.5-4.9 mg/dl	8.5-10.5 mg/dl	male 0.8-1.4, female 0.6-1.2 mg/dl	25-125 U/L	50-212 ug/dl	15-55%	40-200 ng/ml	male 13.4-17.0, female 12.0-15.0	27-34 pg	81-99 fl	<71 pg/ml	<180 RU/ml
1	Male	65	Blue	2.1	9.0	0.8	211	69	19.6%	18.6	11.4	24.0	77	172	557
2	Male	41	Grey	2.5 [a]	9.5	1.0	82.5	83 [a]	22.9%	74.7	16.1	32.3	94	20 [a]	154
3	Female	47	Purple	2.7 [b]	9.7	0.6	93	70 [b]	23.8%	82.5	13.8	30.3	89	68	147
4	Female	25	Green	1.7	9.0	0.7	83	22	5.7%	7.7	10.1	25.4	78	186	498
5	Female	41	Yellow	1.0	8.5	0.6	166	35	8.8%	8.4	12.2	30.7	94	192	540

Graph color refers to the corresponding line on graphs. Subject 4 was on treatment with calcitriol and phosphate for 3 years prior to enrollment. Summary data with median (minimum, maximum) for each study visit are listed in Table 2.

[a]At screening subject 2 qualified based on serum phosphorus of 2.2 mg/dl with iron of 67 ug/dl and intact FGF23 of 42 pg/ml.

[b]At screening subject 3 qualified based on serum phosphorus of 2.5 mg/dl with iron of 48 ug/dl and intact FGF23 of 53 pg/ml..

Table 2.

Group biochemistries throughout the study. Data are shown as median (minimum, maximum).

	Phosphorus	Calcium	Creatinine	Alkaline phosphatase	Iron	Iron saturation	Ferritin	Hemoglobin	Mean cell hemoglobin (MCH)	Mean cell volume (MCV)	Intact Fgf23	C-terminal FGF23
Reference ranges	2.5-4.9 mg/dl	8.5-10.5 mg/dl	male 0.8-1.4, female 0.6-1.2 mg/dl	25-125 U/L	50-212 ug/dl	15-55%	40-200 ng/ml	male 13.4-17.0, female 12.0-15.0	27-34 pg	81-99 fl	<71 pg/ml	<180 RU/ml
Screening	2.2 (1.4, 2.9)	9.3 (8.6, 9.5)	0.8 (0.5, 0.8)	86 (56, 235)	53 (10, 67)	13.8 % (2.6, 24.4)	-	-	-	-	79 (42, 182)	-
Baseline	2.1 (<1.0, 2.7)	9.0 (8.5, 9.7)	0.7 (0.6, 1.0)	93 (83, 211)	69 (22, 83)	19.6 % (5.7, 23.8)	18.6 (7.7, 82.5)	12 (10.1, 16.1)	30.3 (24.0, 32.3)	89 (77, 94)	172 (20, 192)	498 (147, 557)
Month 1	2.5 (1.5, 3.1)	8.8 (8.5, 9.7)	0.7 (0.6, 0.9)	88 (69, 137)	50 (30, 105)	16.9 % (8.6, 34.9)	23.6 (13.7, 102.5)	-	-	-	93 (25, 331)	236 (152, 521)
Month 2	2.5 (1.7, 3.6)	9.1 (8.8, 9.4)	0.6 (0.6, 0.9)	77 (68, 147)	52 (47, 82)	15.7 % (15, 27.9)	47.1 (21.2, 91.4)	-	-	-	54 (22, 72)	152 (116, 219)
Month 3	2.9 (2.6, 3.0)	8.9 (8.5, 9.4)	0.6 (0.6, 1.0)	89 (53, 118)	79 (39, 106)	27 % (13.9, 35.7)	58.5 (41.1, 92.4)	-	-	-	51 (39, 68)	154 (144, 243)
Month 4	3 (2.1, 3.6)	9.2 (9.0, 9.3)	0.7 (0.5, 1.0)	91 (68, 195)	76 (51, 94)	24.5 % (17, 30.5)	46.1 (28, 92.1)	14 (12.8, 15.8)	30.4(29.1, 33.4)	89 (88, 100)	47 (17, 78)	153 (80, 254)
Month 6	2.6 (2.3, 4.2)	9 .0(8.9, 9.2)	0.7 (0.6, 0.9)	95 (60, 157)	71 (35, 133)	22.8 % (11.5, 52)	54.6 (45.8, 127)	14 (13.2, 14.7)	30.1(29.9, 34.2)	91 (88, 101)	27 (16, 45)	146 (67, 172)
Month 8	2.7 (2.5, 4.1)	9.0(8.8, 9.3)	0.7 (0.5, 1.0)	83 (65, 135)	78 (40, 99)	25.4 % (13.1, 34)	59.1 (49.9, 175.1)	-	-	-	56 (25, 82)	158 (95, 169)
Month 10	2.9 (2.7, 3.8)	9.2 (9.0, 9.7)	0.7 (0.6, 0.9)	69 (66, 126)	82 (57, 144)	30.8 % (19.4, 50)	70.8 (39.3, 230.9)	-	-	-	49 (24, 52)	147 (75, 204)
Month 12	2.8 (2.5, 4.0)	9.3 (8.7, 9.5)	0.7 (0.6, 0.9)	69 (60, 120)	82 (43, 117)	29.1 % (14.8, 37.5)	78 (49.6, 261)	14(13.8, 14.5)	30.8 (30.3, 34.3)	91 (88, 102)	42 (19, 63)	146 (121, 175)

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